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Clinical and Experimental Allergy, 1992, Volume 22, pages 391–399

Specificity mapping of patients IgE response towards the tree pollen major allergens *Aln g* I, *Bet v* I and *Cor a* I

H. IPSEN*, J.-Å. WIHL†, B. NÜCHEL PETERSEN‡ and H. LØWENSTEIN*

**ALK Research, Hørsholm, Denmark, †Department of Otorhinolaryngology, University of Lund, Malmö General Hospital, Sweden and ‡Department of Lung Diseases, Copenhagen County Hospital, Gentofte, Denmark*

Summary

The specificity pattern of IgE from non-treated tree pollen allergic patients ($n=38$) were evaluated by solid phase absorption of serum samples followed by CRIE on alder, birch and hazel CIE precipitation profiles. The majority of the serum samples seemed to contain IgE antibodies with the following characteristics: specific towards *Bet v* I alone and common between *Aln g* I, *Bet v* I and/or *Cor a* I, 'II'. The IgE specificity profiles observed for 95% of the sera tested are compatible with birch pollen allergens being the only sensitizing allergens, indicating that the patients react to allergens from other tree pollens of the Fagales order due to IgE cross-reaction with the major allergens of birch and alder and/or hazel pollens.

Clinical and Experimental Allergy, Vol. 22, pp. 391–399. Submitted 16 May 1991; revised 21 October 1991; accepted 25 October 1991.

Introduction

Patients allergic towards a particular allergen source often react towards the taxonomically related species of the same genus, e.g. tree (birch) pollen sensitive patients react towards pollen from many species of the Fagales order [1–6]; grass pollen sensitive patients react towards many of the Poales species [1,7,8] and house dust mite sensitive patients often react towards all three dermatophagoides species [9–12]. Indeed, the major allergens from taxonomically related species exhibit a high degree of similarity both with respect to physico-chemical parameters and immunochemical reactivity towards both poly- and monoclonal antibodies [7,10,12–35]. An increasing number of major allergens have been sequenced completely or in part either by conventional methods or by recombinant DNA techniques [10,23,25, 29,34–45], and the amino acid sequences of allergens from taxonomically related species often exhibit 60–70% sequence identity [10,29,33–41,43] and still higher degrees of sequence homology. This indicates that allergens from related species presumably exhibit epitope patterns which are likely to be more or less preserved within the genus. Therefore, it is probable that the patients concordant sensitivity towards species related allergens originate from congruent or partial identical epitopes. The present paper describes the classification of the specificity of IgE from birch pollen sensitive patients with respect to the cross reaction towards pollen major allergens from the related species alder and hazel.

Correspondence: Dr H. Ipsen, ALK Research, Bøge Allé 10-12, DK-2970. Hørsholm, Denmark.

Materials and methods

Pollen extracts

Aqueous extracts of alder (*Alnus glutinosa*), birch (*Betula verrucosa*) and hazel (*Corylus avellana*) pollens were prepared and handled as described recently [19,29].

Rabbit antibodies

Rabbit anti-alder (a-Ag), anti-birch (a-Bv) and anti-hazel (a-Ca) pollen extract antibodies were raised as described [19,29].

Patient sera

Serum samples ($n=38$) from non-treated patients with seasonal allergy towards pollens from trees of the Fagales order were selected as described in detail elsewhere [2–4].

Immunoelectrophoretic methods

Crossed immunoelectrophoresis [20,29,46] was performed in 1% (w/v) agarose (type HSA, Litex, Denmark)

(0·073 M Tris, 0·024 M barbitale, 0·0006 M calcium lactate pH 8·6) moulded on 5 by 7 cm glass plates. Extracts of alder (Ag, 10 μg), birch (Bv, 15 μg) and hazel (Ca, 10 μg) were applied into wells punched in the agarose at the side measuring 7 cm. First dimension electrophoresis: 15 min for Ag and Bv, 20 min for Ca at 10 V/cm, 15°C; second dimension electrophoresis: 16 hr at 2 V/cm 15°C into separately moulded agarose gels containing 12·5 μl/cm² a-Ag, 16 μl/cm² a-Bv and 11·1 μl/cm² a-Ca rabbit antibodies, respectively. Cold dried crossed immunoplates were incubated with either patient serum (diluted 1:40 (v/v) in 0·027 M Na_2HPO_4, 0·019 M NaH_2PO_4, I = 0·1, pH 7·0) (8 ml) or solid phase absorbed sera (8 ml) (see below) for 16 hr at room temperature, washed three times 10 min each with the dilution buffer and incubated with ^{125}I-anti-IgE [18,47] (approximately 125000 c.p.m.) in 0·027 M Na_2HPO_4, 0·019 M NaH_2PO_4, 0·3% (w/v) bovine serum albumin, 0·154 M NaCl, 0·015 M NaN_3, 0·027 M EDTA pH 7·5 for 16 hr at room temperature. After three 10 min washes (0·154 M NaCl, 1% (v/v) Tween 20), the

Table 1. Listing of the specificities which can account for a radiostaining of a given major allergen. The columns list the specificity combinations allowed after a given solid phase absorption. A, B, C1 and C2 signifies radiostaining of *Aln g* I, *Bet v* I, *Cor a* I and *Cor a* 'II' respectively. Combinations of lettering signifies concordant reactivity

	Ø-Sepharose	Ag-Sepharose	Bv-Sepharose	Ca-Sepharose
Aln g I	ABC1C2			
	ABC1			
	ABC2			
	AC1C2		AC1C2	
	AB			AB
	AC1		AC1	
	AC2		AC2	
	A		A	A
Bet v I	ABC1C2			
	ABC1			
	ABC2			
	BC1C2	BC1C2		
	AB			AB
	BC1	BC1		
	BC2	BC2		
	B	B		B
Cor a I	ABC1C2			
	ABC1			
	AC1C2		AC1C2	
	BC1C2	BC1C2		
	AC1		AC1	
	BC1	BC1		
	C1C2	C1C2	C1C2	
	C1	C1	C1	
Cor a 'II'	ABC1C2			
	ABC2			
	AC1C2		AC1C2	
	BC1C2	BC1C2		
	AC2		AC2	
	BC2	BC2		
	C1C2	C1C2	C1C2	
	C2	C2	C2	

crossed immunoplates were dried and exposed on Kodak XL-1 X-ray film for 3 and 10 days at −20°C using an intensifying screen. SRRID was performed as described elsewhere [48].

Solid phase absorption of patient sera

The solid phase absorption experiments were performed in a manner similar to those described by Lind *et al.* [9].

The extracts Ag, Bv and Ca were each covalently coupled to CNBr activated Sepharose CL 4B (Pharmacia, Sweden) according to the manufactures' recommendations (20 mg extract per g of CNBr activated Sepharose CL 4B).

Solid phase absorption was performed with 38 sera. One millilitre of patient serum was applied on a column containing 2 ml Protein A Sepharose CL 4 B (Pharmacia, Sweden), equilibrated with 0·027 M Na_2HPO_4, 0·019 M NaH_2PO_4, I = 0·1, pH 7·0 and the column was eluted with 7 ml of the same buffer. The IgG depleted patient serum (column eluate, approximately 8 ml) was aliquoted into four 2 ml samples and applied on columns containing 1 ml of either Sepharose CL 4B, Ag-, Bv- or Ca-Sepharose CL 4B equilibrated with 0·027 M Na_2HPO_4, 0·019 M NaH_2PO_4, I = 0·1, pH 7·0. The serum was eluted from each of the extract-Sepharose columns with 6 ml 0·027 M Na_2HPO_4, 0·019 M NaH_2PO_4, I = 0·1, pH 7·0 and the eluates (8 ml) were each incubated with crossed immunoplates as described above. A set of solid phases were used for one serum sample only.

Quantitation of the IgE binding

The radiostaining intensity on the autoradiogaphs of the images of the major allergen (*Aln g* I, *Bet v* I, *Cor a* I and 'II') precipitates was determined by visual comparison with the intensity of the images of the radiostaining on autoradiographs of several (nine) dilutions of ^{125}I-anti-IgE precipitated in SRRID rings [48]. Each radiostained major allergen was assigned a score number, one through nine, corresponding to the SRRID ring exhibiting a radiostaining with approximately equal intensity. The score number represents twofold dilutions of ^{125}I-anti-IgE, where nine is undiluted and one represents a 512 fold dilution.

Theoretical considerations of cross-reactivity

The apparent specificity of the IgE antibodies towards the major allergens may be separated into two different general reactivity patterns: apparent distinct and apparent common specificity. Distinct specificity represents IgE reactivity towards a particular major allergen only and common specificity represents IgE reactivity towards more than one major allergen. Given four major allergens *Aln g* I (A), *Bet v* I (B), *Cor a* I (C1) and *Cor a* 'II' (C2) a total of 15 different specificity patterns can be anticipated: A; B; C1; C2; ABC1C2; ABC1; ABC2; AC1C2; BC1C2; AB; AC1; AC2; BC1; BC2 and C1C2 where a single letter represents a distinct specificity and a letter combination represents a common specificity towards the allergens represented by the letters. Any IgE reactivity representing a subset of the specificity patterns are possible. Table 1 lists the possible subsets which can account for an observed radiostaining of the individual major allergens by the IgE present in the serum sample after a given solid phase absorption. Each serum was assigned a specificity pattern by comparing Table 1 with the CRIE radiostaining profile, of the individual major allergens, obtained with the solid phase absorbed samples.

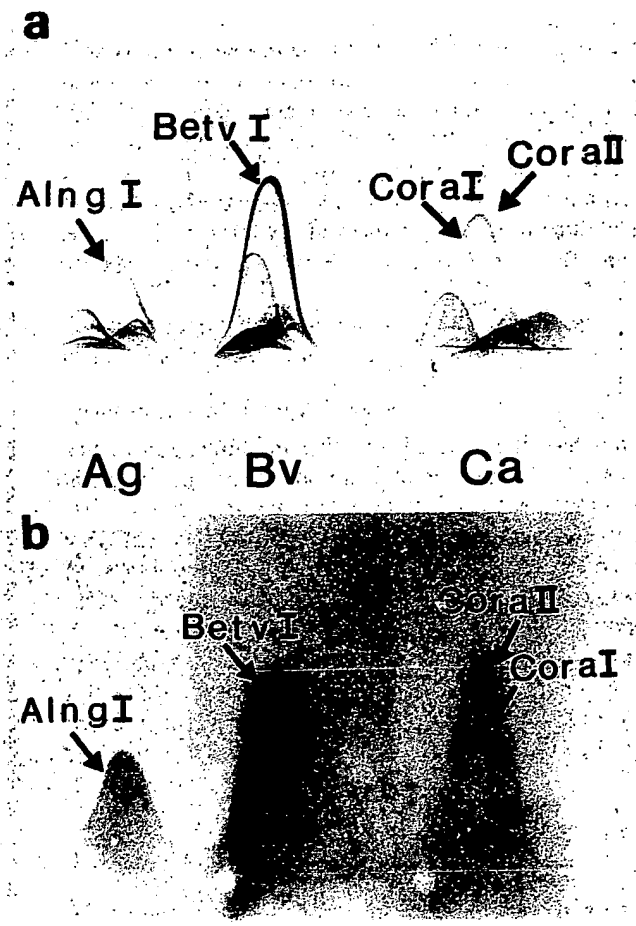

Fig. 1. Crossed immunoelectrophoresis (CIE) precipitation pattern (a) and crossed radioimmunoelectrophoresis (CRIE) autoradiograph (10 days) (b) of alder (Ag), birch (Bv) and hazel (Ca) pollen extracts precipitated with rabbit antibodies raised towards the individual extracts. The major allergens are indicated by arrows on both the CIE and CRIE autoradiograph.

Results

The identity and position of the major allergens *Aln g* I, *Bet v* I, *Cor a* I and *Cor a* 'II' on the CIE plates and CRIE autoradiographs are indicated on Fig. 1. The precipitates of the major allergens are all anodic and although the *Cor a* 'II' precipitate is stained weakly on the CIE immunoplates all the major allergens were clearly visible on the CRIE autoradiographs.

The solid phase absorption procedure for the patient sera is illustrated in Fig. 2, a new set of solid phases (five) were used for each serum sample processed. Examples of the CRIE autoradiographs obtained with solid phase absorbed serum samples are shown in Fig. 3. The CRIE radiostaining intensities and reactivity patterns obtained with untreated serum and serum passed through Protein A- and plain (Ø)-Sepharose CL 4B did not differ significantly (Fig. 3) indicating that the column processing of the sera neither alters the specificity nor the reactivity of the IgE antibodies. Table 2 shows the CRIE score matrixes determined for all ($n=38$) the absorbed serum samples.

The score matrixes can be divided into nine different groups, each representing a unique specificity subset, which occur at widely different frequencies from 16 to 1 out of 38. The specificity pattern of the individual patients IgE was evaluated by comparing the radiostaining of the individual CRIE autoradiographs with the specificity matrix (Table 1), e.g. Fig. 3 and Table 2 serum sample number 8, Table 1. There is a clear radiostaining of *Aln g* I, *Bet v* I, *Cor a* I and *Cor a* 'II' (Fig. 3, Ø-Sepharose, Table 2, column Ø), initially allowing all the specificities tabulated in column Ø, Table 1. Bv-Sepharose abolishes all radiostaining excluding the specificity patterns listed in

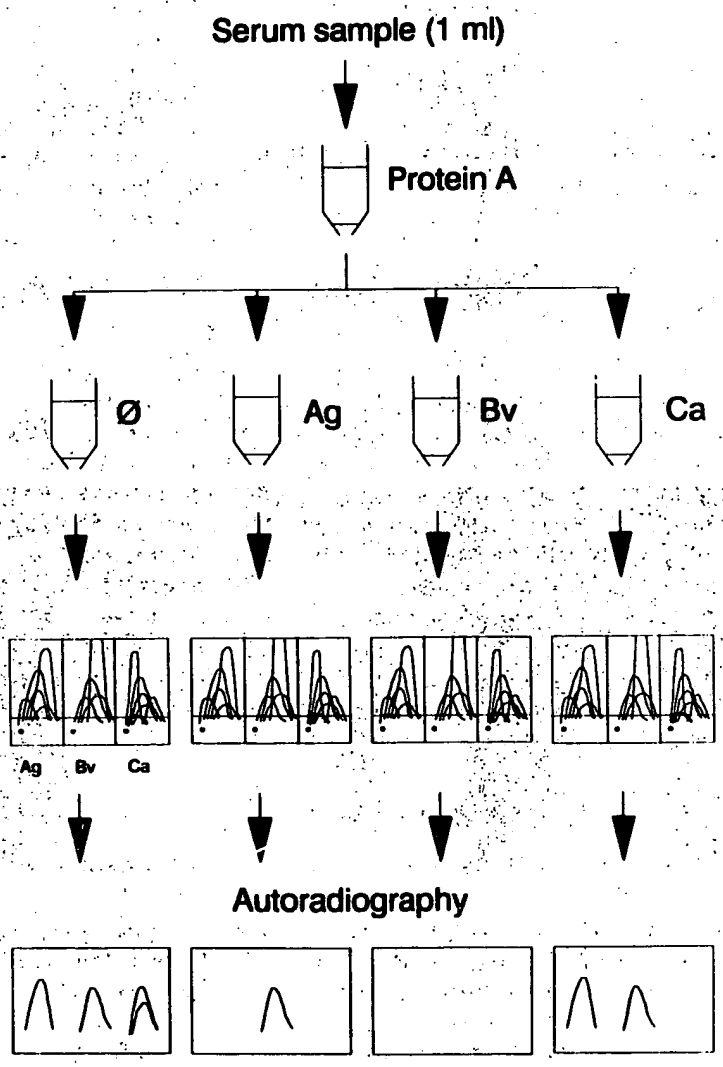

Fig. 2. Schematic representation of the solid phase absorption experiments.

column B, Table 1. Ag-Sepharose allows the radi staining of *Bet v* I only, excluding the specificity patterns listed in column A, Table 1 for *Cor a* I and *Cor a* 'II' indicating distinct B specificity. Ca-Sepharose allows radiostaining of *Aln g* I and *Bet v* I verifying the existence of distinct B and further indicate common AB; distinct A is excluded by the missing radiostaining in column B, row *Aln g* I of Table 1. All the solid phases except Ø-Sepharose removes the radiostaining of *Cor a* I and *Cor a* 'II' indicating IgE with concordant reaction towards all the major allergens. The intensity of the radiostaining (Table 2, serum sample number 8) column Ø > column C > column A further substantiates the presence of common AB but does not exclude common specificity patterns between three or four allergens, leaving serum sample number 8 with the following pattern: B, AB, ABC1, ABC2, ABC1C2.

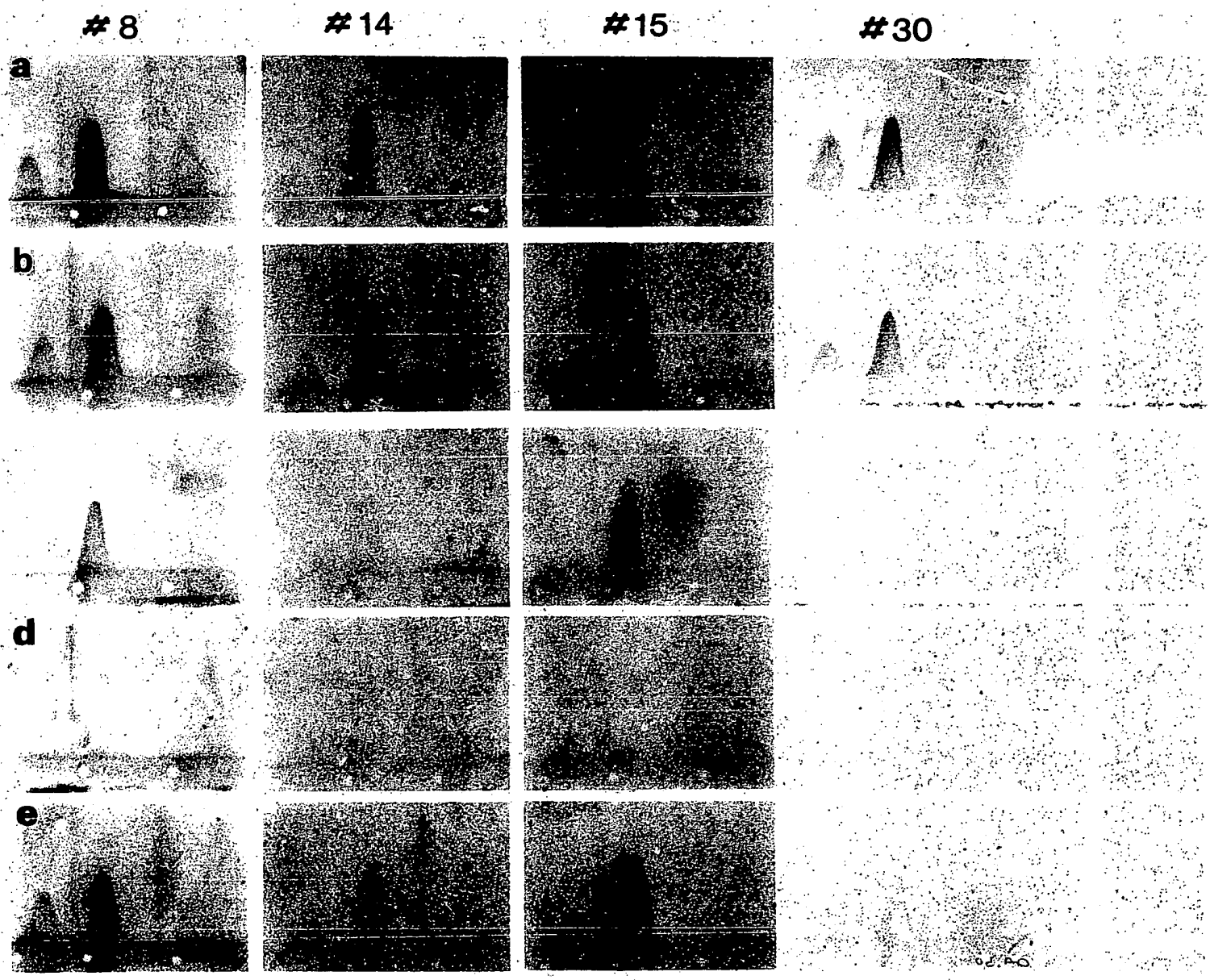

Fig. 3. Examples of CRIE autoradiographs of patient serum samples (numbers 8, 14, 15 and 30) before and after solid phase absorption. The autoradi graphs are marked to indicate the 'treatment' of the serum sample. (a) Untreated serum. (b) Ø-Sepharose. (c) Ag-Sepharose. (d) Bv-Sepharose. (e) Ca-Sepharose.

Table 2. CRIE radiostaining intensity of *Aln g* I, Bet v I, *Cor a* I and *Cor a* 'II' determined with solid phase absorbed serum samples ($n=38$). Ø, plain Sepharose. A, Ag-Sepharose. B, Bv-Sepharose. C, Ca-Sepharose

	#01	Ø	A	B	C	#02	Ø	A	B	C	#03	Ø	A	B	C	#04	Ø	A	B	C
Aln g I		6	0	0	0		5	0	0	5		6	0	0	4		6	0	4	5
Bet v I		7	5	0	5		6	0	0	5		7	5	0	6		8	6	0	7
Cor a I		4	0	0	0		0	0	0	0		6	0	0	0		5	0	0	0
Cor a 'II'		5	0	0	0		0	0	0	0		6	0	0	0		6	0	0	0
	#05	Ø	A	B	C	#06	Ø	A	B	C	#07	Ø	A	B	C	#08	Ø	A	B	C
Aln g I		6	0	0	5		6	0	0	4		6	0	0	5		6	0	0	6
Bet v I		7	5	0	6		7	6	0	6		7	5	0	6		7	6	0	7
Cor a I		5	0	0	0		6	0	0	0		6	0	0	0		5	0	0	0
Cor a 'II'		6	0	0	0		6	0	0	0		6	0	0	0		5	0	0	0
	#09	Ø	A	B	C	#10	Ø	A	B	C	#11	Ø	A	B	C	#12	Ø	A	B	C
Aln g I		6	0	0	6		5	0	0	5		6	0	0	5		5	0	0	4
Bet v I		6	0	0	6		6	6	0	6		7	6	0	6		5	5	0	5
Cor a I		0	0	0	0		5	5	0	0		6	0	0	0		0	0	0	0
Cor a 'II'		5	0	0	0		5	5	0	0		6	0	0	0		0	0	0	0
	#13	Ø	A	B	C	#14	Ø	A	B	C	#15	Ø	A	B	C	#16	Ø	A	B	C
Aln g I		5	0	0	5		4	0	0	4		5	0	0	5		7	0	4	6
Bet v I		6	5	0	6		6	0	0	5		7	6	0	7		7	6	0	7
Cor a I		4	0	0	0		0	0	0	0		0	0	0	0		7	0	0	0
Cor a 'II'		4	0	0	0		0	0	0	0		0	0	0	0		7	0	0	0
	#17	Ø	A	B	C	#18	Ø	A	B	C	#19	Ø	A	B	C	#20	Ø	A	B	C
Aln g I		6	0	0	4		6	0	0	6		5	0	0	4		6	0	0	4
Bet v I		6	0	0	4		6	4	0	6		6	0	0	5		7	6	0	6
Cor a I		3	0	0	0		4	0	0	0		0	0	0	0		5	0	0	0
Cor a 'II'		4	0	0	0		5	0	0	0		0	0	0	0		5	0	0	0
	#21	Ø	A	B	C	#22	Ø	A	B	C	#23	Ø	A	B	C	#24	Ø	A	B	C
Aln g I		6	0	0	5		6	0	0	5		6	0	0	5		5	0	0	4
Bet v I		6	5	0	6		6	3	0	5		6	5	0	5		6	0	0	4
Cor a I		4	0	0	0		4	0	0	0		0	0	0	0		0	0	0	0
Cor a 'II'		5	0	0	0		4	0	0	0		5	0	0	0		0	0	0	0
	#25	Ø	A	B	C	#26	Ø	A	B	C	#27	Ø	A	B	C	#28	Ø	A	B	C
Aln g I		4	0	0	4		5	0	0	0		6	0	0	4		6	0	0	5
Bet v I		4	4	0	5		7	5	0	6		6	0	0	5		6	0	0	5
Cor a I		0	0	0	0		5	0	0	0		5	0	0	0		5	0	0	0
Cor a 'II'		0	0	0	0		5	0	0	0		5	0	0	0		5	0	0	0
	#29	Ø	A	B	C	#30	Ø	A	B	C	#31	Ø	A	B	C	#32	Ø	A	B	C
Alng I		6	0	0	5		7	0	0	5		6	0	0	5		5	0	0	4
Bet v I		6	5	0	6		7	0	0	6		7	0	0	6		7	5	0	7
Cor a I		5	0	0	0		5	0	0	0		4	0	0	0		0	0	0	0
Cor a 'II'		5	0	0	0		6	0	0	0		5	0	0	0		0	0	0	0
	#33	Ø	A	B	C	#34	Ø	A	B	C	#35	Ø	A	B	C	#36	Ø	A	B	C
Aln g I		4	0	0	4		5	0	0	4		5	0	0	4		6	0	0	5
Bet v I		7	5	0	7		6	4	0	5		7	5	0	6		7	5	0	6
Cor a I		3	0	0	0		0	0	0	0		4	0	0	0		4	0	0	0
Cor a 'II'		3	0	0	0		0	0	0	0		4	0	0	0		5	0	0	0
	#37	Ø	A	B	C	#38	Ø	A	B	C										
Aln g I		6	0	0	5		5	0	0	5										
Bet v I		7	4	0	6		5	0	0	5										
Cor a I		6	0	0	0		0	0	0	0										
Cor a 'II'		6	0	0	0		0	0	0	0										

Table 3. The reactivity pattern of 38 patient sera deduced from the s lid phase absorption experiments

Specificity	Number of sera	Percentage of all sera
B, AB, ABC1, ABC2, ABC1C2	16	42·1
AB, ABC1, ABC2, ABC1C2	6	15·8
AB	5	13·2
B, AB	4	10·2
B, ABC1, ABC2, ABC1C2	2	5·3
A, B, AB, ABC1, ABC2, ABC1C2	2	5·3
AB, ABC1	1	2·6
B, BC1, BC2, AB, BC1C2, ABC1, ABC2, ABC1C2	1	2·6
B, AB, ABC2	1	2·6

All the serum samples were analysed in a likewise manner and Table 3 summarizes the specificity patterns deduced and the number of samples which exhibit a given pattern. The pattern observed for sample number 8 was by far the most frequent and only two sera may contain IgE which apart from other specificities exhibit distinct A reactivity. However, this assignment is very uncertain due to the weakness of the radiostaining of *Aln g* I after absorption with Bv and Ca. The specificity patterns ABC1C2, ABC1 and ABC2 cannot be verified in the same way as the other patterns and their existence primarily are deduced from intensity ranking and therefore the patterns are less certain than any of the other patterns observed.

Discussion

The majority of the proteins extractable from pollens of alder, birch and hazel seem to be present as at least immunochemically homologous variants in all the extracts [19,20,29]. The major allergens *Aln g* I, *Bet v* I and *Cor a* I exhibit a degree of similarity with respect to: molecular weight, pI variation, amino acid composition, NH_2-terminal or complete amino acid sequence, hydropathy profile and secondary structure propensity [13–29] which strongly indicate that the fundamental three-dimensional structure of these molecules are the same [49], and the differences between the allergens presumably originate from individual but small perturbations of the general structure.

Rabbit antibodies raised towards isolated tree pollen major allergens exhibit a strong crossreactivity towards the allergens of the related species [19,29]. Although the patients IgE response originate from a fundamentally different immunization scenario, there is no reason to assume that the IgE antibodies should exhibit any restriction with respect to their epitope specificity. Therefore it is likely that the IgE antibodies will be able to react with many, if not all, of the available epitopes on the major allergens and due to the similarity between the allergens, it is probable that the IgE antibodies may crossreact with two or more of the major allergens. Recently Lind *et al.* [9,10] demonstrated that the specificity profile of patients IgE response towards the major allergens of house dust mites, *Der p* I, *Der f* I and *Der m* I exhibit both species specific and common reactivity. Further, Borch *et al.* [15] showed that the IgE response towards *Co a* I and 'II' seem to be crossreactive.

The specificity pattern of the patients IgE, deduced from the solid phase absorption experiments, strongly indicate that the patients' cross sensitivity towards pollens from trees of the Fagales order originate from IgE which react with epitopes common to all the major allergens and/or combinations of reactivity towards epitopes common between at least two major allergens (Table 3). None of the 38 sera exhibited an absorption pattern compatible with an exclusive species specific response. The majority of the sera (68·4%) seemed to contain IgE antibodies which react towards both apparent *Bet v* I specific epitopes and combinations of apparent common epitopes (Table 3). Only two sera, apart from common reactivities, seem to exhibit specific reactivity towards an allergen (*Aln g* I) different from *Bet v* I (Table 3). Further, all the common specificity patterns observed included a reactivity towards *Bet v* I, i.e. IgE reacting with *Bet v* I and at least one of the other major allergens, indicating that birch pollen are the primary, if not the only, sensibilization source for these patients. Clinical data, i.e. hyposensitization, of these patients with extracts of either birch pollen or combinations of alder, birch and hazel pollen extracts seem to substantiate the above conclusion, since treatment with birch pollen extract alone provide the same clinical efficacy as did the mixture of the extracts [2–4].

Acknowledgment

The skilful technical assistance of Annette Giselsson is gratefully acknowledged.

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